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CONTRACTING ORGANIZATION: University of Chicago
Chicago, IL 60637

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## Abstract

Several groups have demonstrated that women with *BRCA1* mutations are more likely to have breast cancers that are hormone receptor and HER2/*neu* negative. Our lab has previously demonstrated that *BRCA1* promoter methylation occurs to some degree in 30% of all sporadic tumors, and up to 50% of high-grade hormone receptor negative tumors, making it much more common than germline mutation. Given the role of *BRCA1* in DNA repair, it is likely that cells deficient in BRCA1 secondary to promoter methylation will have an increased sensitivity to DNA damaging agents. The role of *BRCA1* methylation in determining chemosensitivity is not yet known. Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are more sensitive to cisplatin than *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary promoter methylation are also highly sensitive to cisplatin. As *BRCA1* methylation occurs in almost one-half of high-grade hormone receptor negative tumors, it represents a potential therapeutic target in the treatment of a subset of hormone receptor negative breast cancers.

## INTRODUCTION

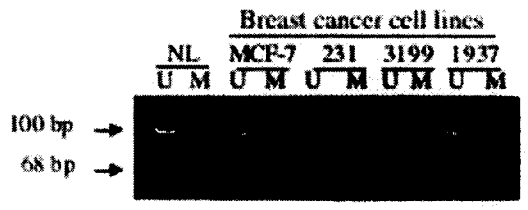
Several groups have demonstrated that women with *BRCA1* mutations are more likely to have breast cancers that are hormone receptor and HER2/*neu* negative[1]. Our lab has previously demonstrated that *BRCA1* promoter methylation occurs to some degree in 30% of all sporadic tumors, and up to 50% of high-grade hormone receptor negative tumors, making it much more common than germline mutation[2]. Given the role of *BRCA1* in DNA repair, it is likely that cells deficient in BRCA1 secondary to promoter methylation will have an increased sensitivity to DNA damaging agents, as has previously been demonstrated in cells deficient in BRCA1 secondary to mutation[3, 4]. The role of *BRCA1* methylation in determining chemosensitivity is not yet known. As *BRCA1* methylation occurs in almost one-half of high-grade hormone receptor negative tumors, it represents a potential therapeutic target in the treatment of a subset of hormone receptor negative breast cancers.

## BODY

**Task 1**: To test *BRCA1* normal, *BRCA1* mutated and *BRCA1* methylated breast cancer cell lines *in vitro* for sensitivity to chemotherapeutic agents commonly employed in breast cancer treatment.

A). Confirm *BRCA1* mutated or methylated status of cell lines of interest.
The UACC-3199 cell line was established at the University of Arizona Cancer Center. This *BRCA1* promoter region of this cell line has been studied, and is know to be extensively methylated[5]. Prior to proceeding with cytotoxicity assays, the *BRCA1* status of all cell lines used was confirmed. The UACC-3199 cells is known to be *BRCA1* methylated, and the HCC-3199 cell line is known to have one copy of a mutated *BRCA1* gene. MDA-MB-231 (MB-231) and MCF-7 are known to have normal *BRCA1*. To confirm that the BRCA1 promoter region was methylated in the UACC-3199 cell line and not in the other cell lines, methylation specific PCR (MSP) was performed on bisulfite converted cell line DNA. MSP was performed as previously described[6]. Primer sequences for the methylated product were 5'-TCG TGG TAA CGG AAA AGC GC-3' (sense) and 5'-AAC GAA CTC ACG CCG CGC AA-3' (antisense) and for the unmethylated product were 5'-TTG AGA GGT TGT TGT TTA GTG G-3' (sense) and 5'-AAC AAA CTC ACA CCA CAC AA-3' (antisense). The methylated product is 68 bp in length and the unmethylated product is 100 bp in length, with both containing the transcription initiation site. As expected and shown in figure one below, the UACC-3199 cell line was methylated for the BRCA1 promoter (as evidenced by a 68 bp product in the methylated lane). The other three cell lines were all confirmed to be unmethylated, as evidenced by the 100 bp product in the unmethylated lanes). Normal human lymphocytes served as the unmethylated control.

**Figure 1: MSP for BRCA1**




BRCA1

Next, Western blot analysis was performed on protein lysates extracted from the cell lines studied as previously described[6]. Two antibodies were used to detect the BRCA1 protein, the C-20 antibody, which binds to the carboxy terminus of the protein, and D-20, an antibody that binds to the amino terminus of the protein. As demonstrated in figure 2, the UACC-3199 cell line has decreased BRCA1 expression in comparison with the MB-231 and MCF-7 cell lines, as expected. No *BRCA1* expression for the HCC-1937 cell line is detected, as the protein expression in this cell line is truncated. In figure 3 below, using the antibody directed to the N-terminus, abundant truncated protein expression is detected in the HCC-1937 cell line. The *BRCA1* methylated UACC-3199 cell line again, demonstrates decreased expression as compared to the normal MCF-7 cell line.

**Figure 2: Western Blot analysis for *BRCA1* expression using antibody to C-terminus**

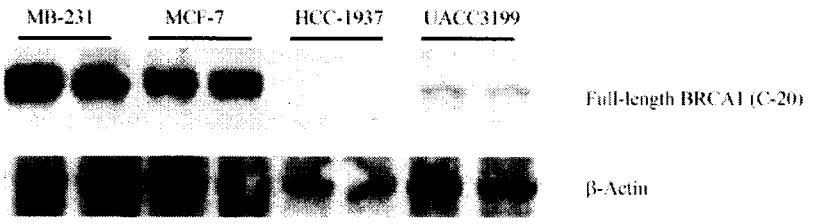
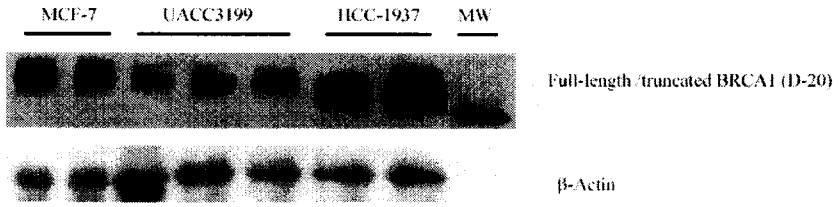



**Figure 3: Western Blot analysis for *BRCA1* expression using antibody to N-terminus**




MSP and Western blot analysis confirmed the presence of extensive *BRCA1* promoter methylation of the UACC-3199 cell line and the presence of a truncated BRCA1 protein in the HCC-1937 cell line, consistent with the literature.

B). Perform *in vitro* sensitivity assays with various chemotherapeutic agents of interest.

Using an *in vitro* cell line model, the relative sensitivity of *BRCA1* methylated, mutated and competent breast cancer cell lines was determined. Four breast cancer cell lines were used to determine relative sensitivity: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with doses of cisplatin between 0.25 uM and 350 uM. Each cell line was exposed to escalating doses of cisplatin in triplicate on three separate times. Untreated cells served as a control. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival and apoptosis were determined by flow cytometry using FACS DiVa. FlowJo FACS analysis software (version 6.1.1) was used to

generate percent apoptotic and live cells. Cells that were negative for both Annexin-V and DAPI were considered live, and cells that were positive for Annexin-V and negative for DAPI were considered apoptotic. Each experiment was normalized to its own dose 0 average, and percent live vs dose and percent apoptotic vs dose curves were constructed. $IC_{50}$ values were calculated from sigmoidal dose response curves. Results are in table 1 below. Plots of dose response curves can be found in Appendix I.

Table 1: $IC_{50}$ and Peak % Apoptotic Cells by cell line after exposure to Cisplatin

| Cell Line | $IC_{50}$ for Cisplatin (uM) |
|---|---|
| UACC-3199 | 16.7 |
| HCC-1937 | 78.0 |
| MDA-MB-231 | > 350 |
| MCF-7 | > 350 |

| Cell Line | Peak % Apoptosis | Concentration Cisplatin (uM) |
|---|---|---|
| UACC-3199 | 40 | 50 |
| HCC-1937 | 20 | 100 |
| MDA-MB-231 | 16 | 350 |
| MCF-7 | 21 | 350 |

Cytotoxicity and apoptosis assays using other chemotherapies commonly used in the treatment of breast cancer are ongoing.

C). Perform synergy assays.
Due to delays in optimization of *in vitro* cytotoxicity assays, synergy assays have not yet begun.

**Task 2:** To explore the role of *BRCA1* methylation in hormone receptor negative tumors and how it might affect response to chemotherapy *in vivo*.

A). Write protocol for gemcitabine/cisplatin in metastatic breast cancer
B). IRB approval
C). Accrue patients to study

Unfortunately, secondary to poor accrual, the gemcitabine/cisplatin trial has been closed. The trial was open for eight months, and did not accrue any patients. The main reason for poor accrual was that as both gemcitabine and cisplatin are commercially available, and many patients opted for treatment off protocol with a local oncologist. While the trial was also open to other centers that are part of the University of Chicago Phase II Network, many of the physicians did not enroll patients to this trial, as they did not feel that this regimen offered their patients a "novel" treatment. Furthermore, many community oncologists were reluctant to give cisplatin, given the length of time the treatment takes (1 hour of pre-treatment hydration and 1 hour of post-treatment hydration).

To address the factors that resulted in our inability to accrue to the previous study, another trial has been designed to replace it. The goal is the same: to explore the role of *BRCA1* methylation and correlate methylation with response to DNA-damaging based chemotherapy. Given the concerns regarding cisplatin use, carboplatin will be used instead. To address the concerns that the trial did not use novel agents, bevacizumab has been added. Based on my mentor's ongoing collaboration with Charles Perou, Ph.D., from the University of North Carolina-Chapel Hill, we know from microarray data that hormone receptor negative tumors have high levels of angiogenesis (personal communication). Given that almost 50% these tumors are also likely to

have some degree of *BRCA1* promoter methylation, a combination of an antiangiogenic agent and a DNA damaging agent will be an effective and novel combination in the treatment of these aggressive tumors. I attended the 2004 AACR/ASCO Methods in Clinical Cancer Research Workshop, where I designed and developed a protocol based on these principles. For the complete protocol and consent, see Appendix II.

## KEY RESEARCH ACCOMPLISHMENTS

Task 1

- Abstract based on *in vitro* data submitted for 2005 San Antonio Breast Conference (see Appendix III)

Task 2

- Formal training completed in protocol design and development at 2004 AACR/ASCO Methods in Clinical Cancer Research Workshop
- Peer-reviewed protocol developed at AACR/ASCO workshop will be basis of future grant submission

## REPORTABLE OUTCOMES

- Based on my ability to secure research funding from the Department of Defense, I have been offered a position as an Instructor in the Department of Medicine, Section of Hematology/Oncology at the University of Chicago. I will have 90% protected time to devote to my research program and development.

## CONCLUSIONS

Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are more sensitive to cisplatin than *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary promoter methylation are also highly sensitive to cisplatin. As *BRCA1* methylation occurs in almost one-half of high-grade hormone receptor negative tumors, it represents a potential therapeutic target in the treatment of a subset of hormone receptor negative breast cancers. Our ability to identify novel "targets" for this subset of aggressive cancers will allow us to maximize efficacy while minimizing side effects from therapy, with the goal of allowing these women to live longer and better.

## REFERENCES


1. Grushko, T.A., et al., *Molecular-cytogenetic analysis of HER-2/neu gene in BRCA1-associated breast cancers.* Cancer Res, 2002. **62**(5): p. 1481-8.
2. Wei, M., et al., *Hypermethylation of BRCA1 promotor in sporadic breast cancer: comparison with BRCA1-associated hereditary breast cancer.* Breast Cancer Research and Treatment, 2002. **76**: p. S35.
3. Tassone, P., et al., *BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells.* Br J Cancer, 2003. **88**(8): p. 1285-91.
4. Quinn, J.E., et al., *BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis.* Cancer Res, 2003. **63**(19): p. 6221-8.
5. Rice, J.C., K.S. Massey-Brown, and B.W. Futscher, *Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells.* Oncogene, 1998. **17**(14): p. 1807-12.
6. Grushko, T.A., et al., *MYC is amplified in BRCA1-associated breast cancers.* Clin Cancer Res, 2004. **10**(2): p. 499-507.

# APPENDIX I

**Figure 4: Percent Live Cells vs Cisplatin Concentration (uM) for UACC-3199 Cell Line**

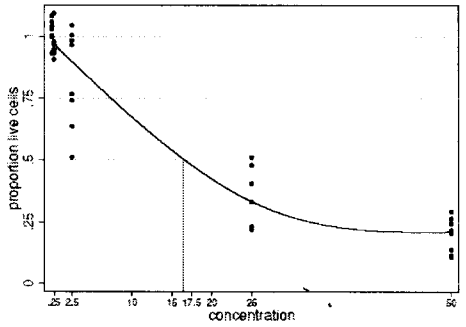



**Figure 5: Percent Live Cells vs Cisplatin Concentration (uM) for HCC-1937 Cell Line**

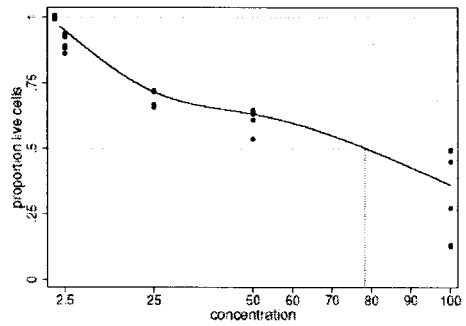



**Figure 6: Percent Live Cells vs Cisplatin Concentration (uM) for MDA-MB-231 Cell Line**

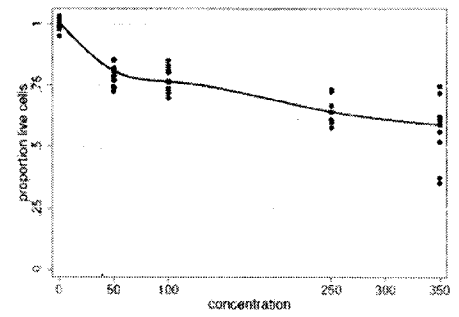



Figure 7: Percent Live Cells vs Cisplatin Concentration (uM) for MCF-7 Cell Line

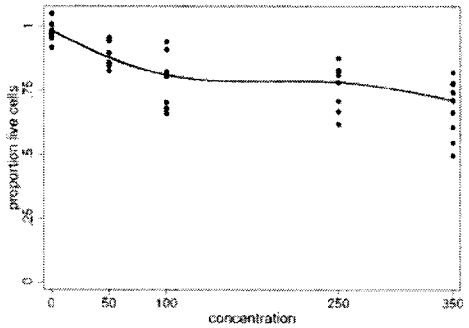


**Figure 8: Percent Apoptotic Cells vs Cisplatin Concentration (uM) for UACC-3199 Cell Line**

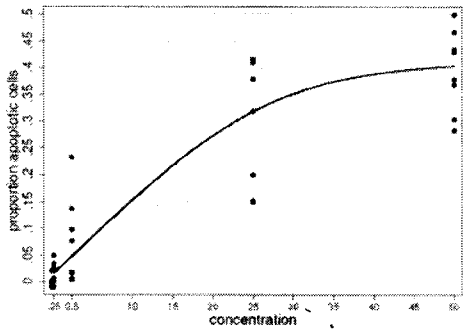



**Figure 9: Percent Apoptotic Cells vs Cisplatin Concentration (uM) for HCC-1937 Cell Line**

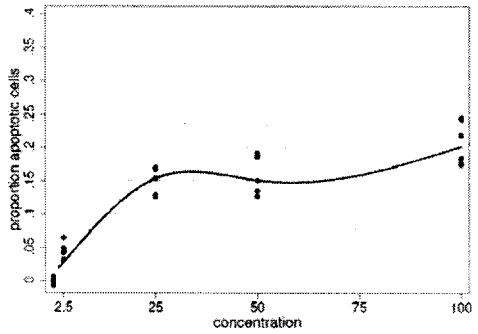



**Figure 10: Percent Apoptotic Cells vs Cisplatin Concentration (uM) for MDA-MB-231 Cell Line**

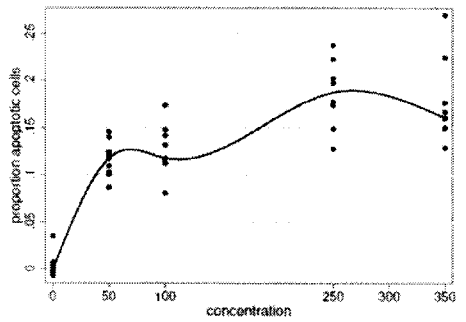
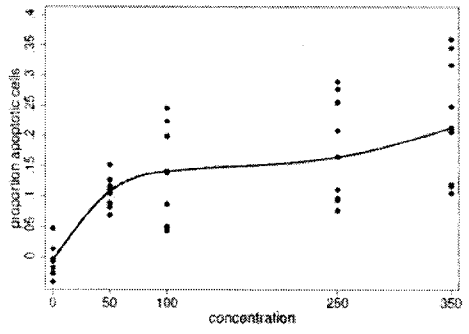



**Figure 11: Percent Apoptotic Cells vs Cisplatin Concentration (uM) for MCF-7 Cell Line**



# APPENDIX II

American Association for Cancer Research

ASCO
AMERICAN SOCIETY OF CLINICAL ONCOLOGY

# 2004 AACR/ASCO WORKSHOP
# Methods in Clinical Cancer Research

**July 31-August 6, 2004 • Vail Marriott Mountain Resort • Vail, Colorado**

September 7, 2004

Rita Nanda, M.D.
PostdoctoralFellow, Dept. of Hematology/Oncology
Univ.of Chicago Medical Ctr.
5841S. Maryland Ave., MC 2115
Chicago, IL 60637
UNITEDSTATES

Dear Dr.Nanda:

Congratulations on your successful completion of the course requirements for the *2004 AACR/ASCO Methods in Clinical Cancer Research Workshop*. Enclosed you will find your certificate of completion for the protocol you submitted in connection with this summer's Workshop. As organizers of the Workshop, AACR and ASCO would like to hear from you!

The Program Committee that is planning next summer's Workshop is most interested in having your post-conference views and constructive comments to help make the next program even better. While we will be mailing a more formal evaluation document in a few months, your letter now (or an e-mail to mendenhall@aacr.org) with your impressions, the status of the protocol you developed during the course, and any other suggestions would be most appreciated.

We look forward to hearing from you and wish you good luck on your protocol implementation. Thank you for taking the time to help us make this Workshop an even more effective training experience for future cancer researchers.

Sincerely,

Margaret Foti, Ph. D., M.D. (h.c.)
Chief Executive Officer, AACR

Enc.

CONFERENCE SECRETARIATS:

**AMERICAN ASSOCIATION FOR CANCER RESEARCH**
615 Chestnut Street • 17th Floor • Philadelphia, PA 19106
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**AMERICAN SOCIETY OF CLINICAL ONCOLOGY**
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**Telephone: (703) 299-0150 • Fax: (703) 299-1044 • Website: www.asco.org**

American Association for Cancer Research

ASCO
AMERICAN SOCIETY OF CLINICAL ONCOLOGY

# CERTIFICATION

AACR/ASCO Workshop "Methods in Clinical Cancer Research"
Vail, Colorado, July 31 – August 6, 2004

This is to certify that

## *RITA NANDA, M.D.*

has received special training in the design and conduct of clinical trials by successfully completing the design and preparation of a clinical trial protocol entitled:

*A PHASE II STUDY OF CARBOPLATIN AND BEVACIZUMAB +/- TRASTUZUMAB COMBINATION THERAPY IN PATIENTS WITH HORMONE RECEPTOR NEGATIVE METASTATIC BREAST CANCER*

Daniel D. Von Hoff, M.D.
Course Director

Joel E. Tepper, M.D.
Course Director

Susan G. Hilsenbeck, Ph.D.
Course Director

**A Phase II Study of Carboplatin and Bevacizumab +/- Trastuzumab Combination Therapy in Patients with Hormone Receptor Negative Metastatic Breast Cancer**


| | |
|---|---|
| **Coordinating Center:** | University of Chicago Phase II Consortium |
| ***Principal Investigator:** | Rita Nanda, M.D.<br>University of Chicago Medical Center<br>5841 S. Maryland Ave, MC 2115<br>Chicago, IL 60637<br>Phone: 773-834-2756<br>Fax: 773-702-3163 |
| **Co-Investigators:** | Funmi Olopade, M.D.<br>Gini Fleming, M.D. |
| **Statistician:** | James Dignam, Ph.D. |
| **Responsible Research Nurse:** | Maureen Maple, R.N. |
| **Responsible Data Manager:** | Bernadette Libao |

## SCHEMA

### A Phase II Study of Carboplatin and Bevacizumab +/- Trastuzumab Combination Therapy in Patients with Hormone Receptor Negative Metastatic Breast Cancer

| Patient Eligibility | Required Laboratory Data |
|---|---|
| Pathologically confirmed metastatic breast cancer . | Granulocytes ≥ 1,500/uL |
| Tumor is hormone receptor negative (ER and PR negative). | Platelets ≥ 100,000/uL |
| Measurable disease. | Bilirubin ≤ ULN[1] |
| ECOG PS 0-2. | AST and ALT ≤ 2.5 ULN |
| Age ≥ 18 years. | Creatinine (Cr) ≤ ULN |
| Non-pregnant and not breast feeding. | Creatinine Clearance ≥ 60 mL/min/1.73m2 (if Cr above ULN) |
| No prior therapy for metastatic disease. Greater than 4 weeks since last dose of adjuvant chemotherapy or radiotherapy. | PT INR ≤ 1.5[2] |
| No prior therapy with platinum agents, trastuzumab, bevacizumab, or other VEGF inhibitors. | Urine protein = none[3] |
| No evidence of CNS disease, including primary brain tumor. | |
| No currently active secondary malignancy. | |
| No prior history of significant bleeding (see section 3.2.9). | |
| Tumor block must be available for correlative studies | |
| Peripheral neuropathy ≤ grade 1 | |
| No other serious medical or psychiatric disease. | |
| No serious or non-healing wound, ulcer or bone fracture. | |
| No serious active infection (viral, fungal, bacterial). No infection requiring parenteral antibiotics at the time of registration. | |
| No clinically significant cardiac disease (see section 3.2.5). | |
| No inadequately controlled hypertension (see section 3.2.10) | |

[1]ULN = upper limits of normal

[2]Unless patient is on anticoagulation. Pt must be on a stable dose of warfarin or LMWH and have an in-range INR (usually between 2-3).

[3]For ≥ 1+ proteinuria on urine dipstick, if a 24 hour urine collection demonstrates ≤ 1gm/dL of protein/24 hours the patient would be eligible.

## TREATMENT PLAN

Patients with hormone receptor negative breast cancer who have not received prior chemotherapy for metastatic disease (they may have received adjuvant chemotherapy), will be treated on one of two arms, depending on their HER2/*neu* status.

Arm 1: HER2/*neu* negative patients
(defined as HER2/*neu* 0-1+ by IHC or FISH negative for gene amplification)*
Carboplatin AUC 6 IV every 3 weeks
Bevacizumab 10 mg/kg IV every 3 weeks

Arm 2: HER2/*neu* positive patients
(defined as HER2/*neu* 3+ by IHC or FISH positive for gene amplification)*
Carboplatin AUC 6 IV every 3 weeks
Bevacizumab 10 mg/kg IV every 3 weeks
Trastuzumab 8mg/kg loading dose and 6 mg/m2 every 3 weeks thereafter

*Note: Tumors that are 2+ for HER2/*neu* by IHC must have FISH performed prior to enrollment

**Correlative Studies**: Tumor blocks from the primary tumor will be collected on all women and examined for *BRCA1* promotor methylation. Tissue Microarrays (TMA) will be constructed for further immunohistochemical study of markers of interest.

**Potential Toxicity**: Nausea, vomiting, fatigue, dyspnea, neurotoxicity, ototoxicity, renal dysfunction, electrolyte wasting, rash, bleeding, thrombotic events, cardiotoxicity, hypertension.

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## 1. OBJECTIVES

1.1. Primary Objective

To determine the response rate of metastatic hormone receptor negative breast cancers to the combination of carboplatin and bevacizumab therapy (and trastuzumab for Her2/*neu* amplified tumors).

1.2. Secondary Objectives

1.2.1. To determine the time to disease progression.

1.2.2. To perform a preliminary analysis exploring whether tumors that have *BRCA1* promoter hypermethylation have a response rate that differs from those tumors without *BRCA1* promoter hypermethylation.

1.2.3. To perform a preliminary analysis exploring whether response can be correlated to tumor VEGF expression.

## 2. BACKGROUND

2.1 **Metastatic Breast Cancer**

Breast cancer is the second highest cause of cancer death in women in the United States. While breast cancer is being diagnosed at earlier stages because of mammography, approximately 20-85% of patients go on to develop distant metastases. About 6-10% of women in the U.S. present with metastatic disease. Chemotherapy and hormone therapy have both been used in the treatment of metastatic disease. Most patients experience an objective response, with responses lasting anywhere from 8-14 months. Unfortunately, progression of disease is inevitable, and response to second-line therapy is less likely. While a few trials for certain first-line regimens are associated with improved survival, chemotherapy beyond first-line treatments has not been shown to improve overall survival. Furthermore, hormone receptor negative and HER2/*neu* amplified breast cancers have both been associated, independent of stage at presentation, with a higher risk of relapse as compared to ER positive tumors.

2.2 **Role of *BRCA1* in Sporadic Breast Cancers**

Germline mutations in *BRCA1* and *BRCA2* are thought to account for 5-10% of breast cancer cases. While *BRCA1* germline mutations are relatively uncommon, epigenetic alterations in *BRCA1* are much more common. Work in the laboratory of Dr. Funmi Olopade at the University of Chicago has demonstrated that almost 50 % of women with ER negative breast cancers tumors are likely to have some degree of hypermethylation of the *BRCA1* promoter region of their tumors. Hypermethylation would result in decreased to absent brca1 protein expression. *BRCA1* and *BRCA2* have both been implicated in DNA damage responses including double-strand break repair. It has been hypothesized that cells deficient in *BRCA1* or *BRCA2* may have increased sensitivity to ionizing radiation and DNA damaging chemotherapy, such as cisplatin. It has been suggested that this increased platinum sensitivity accounts for the increased survival of ovarian cancer patients with BRCA mutations as most ovarian cancer patients are treated

with platinum-containing regimens as part of their first-line chemotherapy. There are also convincing *in vitro* data demonstrating that *BRCA1* deficient cells are sensitive to platinum therapy and resistant to paclitaxel, which is currently one of the most commonly used chemotherapies in the treatment of breast cancer.

2.3 **Platinum agents and Breast Cancer**

As a single agent, carboplatin has been reported to have about a 30% response rate in previously untreated breast cancer and a response rate of less than 10% in women with previous therapy. However, there has recently been increased interest in using platinum agents for women with breast cancer because of the marked synergy suggested *in vitro* with carboplatin and trastuzumab, and promising preliminary results with cisplatin and trastuzumab in heavily pretreated breast cancer patients. A cisplatin/docetaxel regimen and a carboplatin/paclitaxel regimen have both been shown to have good activity as first-line treatment for breast cancer and the carboplatin /docetaxel regimen is currently being tested in the adjuvant setting. Cisplatin causes more nausea, vomiting, and neuropathy that carboplatin, but less myelotoxicity. Overall, carboplatin appears to be as least as effective as cisplatin, and much better tolerated.

2.4 **VEGF Inhibitors and Breast Cancer**

Hormone receptor and HER2/*neu* negative tumors are highly angiogenic and overexpress VEGF. HER2/*neu* positive tumors are similarly highly angiogenic and have high levels of VEGF expression. Bevacizumab, a VEGF inhibitor that has demonstrated a significant improval in overall survival in colorectal cancer, was administered to women with metastatic breast cancer. In a heavily pretreated cohort of patients, bevacizumab monotherapy demonstrated a 9% response rate. In a randomized phase III trial of capecitabine vs capecitabine/bevacizumab in women with metastatic breast cancer, the bevacizumab/capecitabine arm failed to show any improvement over capecitabine monotherapy. However, these patients were not selected for VEGF overexpression, and the effect of the addition of bevacizumab in this subset of patients could have been masked.

2.5 **Rationale for Study**

In this trial, we propose to study the effects of carboplatin and bevacizumab (+/- trastuzumab) combination therapy in a cohort of women who are highly likely to respond to these therapies based on the biology of their tumors. Because they are highly likely to have some degree of brca1 deficiency secondary to *BRCA1* promoter methylation and tumors with high levels of VEGF expression, they are highly likely to respond to the combination therapy we propose. By "targeting" these abnormalities, we hope to identify a minimally toxic and highly effective therapy for this subset of difficult to treat patients.

## 3. PATIENT SELECTION

### 3.1 Eligibility Criteria

3.1.1 Patients must have pathologically confirmed hormone receptor negative breast cancer (ER and PR negative).

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan. See section 9.2 for the evaluation of measurable disease.

3.1.3 No prior chemotherapy for metastatic breast cancer. Patients may have received adjuvant chemotherapy, and should be ≥ 4 weeks from their most recent chemotherapy or radiation therapy treatment.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of cisplatin in combination with bevacizumab in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 2 combination trials.

3.1.5 ECOG performance status ≤2 (Karnofsky ≥60%; see Appendix A).

3.1.6 Patients must have normal organ and marrow function as defined below:

| | |
|---|---|
| - absolute neutrophil count | ≥1,500/uL |
| - platelets | ≥100,000/uL |
| - total bilirubin | within normal institutional limits |
| - AST(SGOT)/ALT(SGPT) | ≤2.5 X institutional upper limit of normal |
| - creatinine | within normal institutional limits |
| OR | |
| - creatinine clearance | ≥60 mL/min/1.73 $m^2$ for patients with creatinine levels above institutional normal |
| - PT INR | ≤ 1.5* |
| - urine protein | none** |

* Unless patient is on anticoagulation, in which case the patient should be on a stable dose of warfarin or LMWH, with an INR in the desired range (usually between 2-3).

**For patients with proteinuria on urine dipstick, if a 24 hour urine collection demonstrates ≤1 gm/dL of protein, the patient is eligible.

3.1.7 Tissue from the primary tumor must be available for correlative studies.

3.1.8 The effects of bevacizumab, cisplatin and trastuzumab on the developing human fetus at the recommended therapeutic dose are unknown. For this

reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Nursing patients must discontinue breast feeding prior to the initiation of therapy.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.2 **Exclusion Criteria**

3.2.1 Patients who have had prior therapy with platinum agents and bevacizumab are not eligible. Patients who have received trastuzumab as part of their adjuvant therapy are eligible.

3.2.2 Patients may not be receiving any other investigational agents.

3.2.3 Patients with known brain metastases will be excluded from this clinical trial because of the risk of CNS bleeding in patients receiving bevacizumab.

3.2.4 Patients may have had prior radiation therapy, provided the patient has measurable disease outside of the radiation port. Patients who have had radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events due to therapy administered more than 4 weeks earlier will be excluded.

3.2.5 Patients with significant cardiac dysfunction will be excluded from this trial as trastuzumab and bevacizumab are both associated with an increase in the risk of cardiac dysfunction. This includes patients with an ejection fraction below institutional limits of normal, myocardial infarction or unstable angina within 6 months of registration, New York Heart Association grade II or greater CHF, or grade II or greater peripheral vascular disease.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Pregnant women are excluded from this study because carboplatin, bevacizumab and trastuzumab all have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued.

3.2.8 Because patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy, HIV-positive

patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with carboplatin or the other agents administered during the study.

3.2.9 Patients with a history of significant bleeding events will be excluded from this trial, as bevacizumab has been associated with an increased risk of thrombosis and bleeding. This includes patients who have had significant bleeding events within 6 months of registration (e.g. hemoptysis, upper or lower GI bleed). If a patient is on full-dose anticoagulation, the patient must be on a stable dose of warfarin or LMWH and have an in-range INR (usually between 2-3).

3.2.10 Patients with inadequately controlled hypertension will be excluded from this trial as bevacizumab has been associated with hypertensive crisis. Inadequately controlled hypertension is defined as blood pressure > 150/90 on medication.

### 3.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. However, because of the rarity of breast cancer in males, coupled with the fact that most male breast cancers are hormone receptor positive, it is expected that only female patients will be enrolled. The proposed study population is illustrated in the table below. Figures are based on a 5-year average of University of Chicago accrual to CALGB protocol.

| | **Race/Ethnicity** | | | | | |
|---|---|---|---|---|---|---|
| **Gender** | White, not of Hispanic Origin | Black, not of Hispanic Origin | Hispanic | Asian or Pacific Islander | Unknown | Total |
| Male | 0 | 0 | 0 | 0 | 0 | 0 |
| Female | 47 | 21 | 2 | 2 | 0 | 72 |
| Total | 47 | 21 | 2 | 2 | 0 | 72 |

## 4. TREATMENT PLAN

### 4.1 Agent Administration

4.1.1 Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for carboplatin, bevacizumab and trastuzamab are described in Section 6. Appropriate dose modifications are described in Section 5. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

4.1.2 Carboplatin will be administered at a dose of AUC 6 in 250 mL normal saline intravenously over 30 mins. Bevacizumab will be administered at a dose of 10 mg/kg in 100 mL normal saline intravenously over 90 minutes for the first dose and 60 minutes for each subsequent dose. Trastuzumab will be administered at a loading dose of 8 mg/kg over 90 minutes, and then at 6 mg/kg over 60 minutes for each subsequent dose.

4.1.3 Carboplatin will be administered on day 1 of every 3 week cycle. Bevacizumab will be administered on day 1 of every 3 week cycle. A loading dose (8 mg/kg) of trastuzumab will be administered on day 1 of cycle 1 and then at standard dose (6 mg/kg) every 3 weeks thereafter for those patients with HER2/*neu* overexpression).

## 4.2 Supportive Care Guidelines

4.2.1 While the exact regimen to be used is left to the discretion of the treating physicians, all patients should receive anti-emetic therapy. A reasonable option would be a combination of dexamethasone 10-20 mg and a 5HT3 receptor antagonist prior to carboplatin.

4.2.2 The use of darbopoeitin or erythropoeitin is permitted, and should be documented.

4.2.3 The use of granulocyte colony stimulating agents should only be used if there is persistent neutropenia despite a dose reduction in the previous course. G-CSF may also be used as clinically indicated for neutropenic infection.

4.2.4 The use of bisphosphonates is permitted, however in these patients bone should not be used as a site in determining progression of disease.

## 4.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until:

X Disease progression,
X Intercurrent illness that prevents further administration of treatment,
X Unacceptable adverse events(s),

X Patient decides to withdraw from the study, or

X General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Should the patient have a complete response, it is recommended that treatment be discontinued, with two cycles being administered as consolidation at the discretion of the investigator. The decision as to whether to continue in the face of stable disease is left to the choice of the investigator and the patient.

## 5. DOSING DELAYS/DOSE MODIFICATIONS

### 5.1 Hematologic Toxicity

5.1.1 Subsequent cycles of therapy (day 1) will not begin until the ANC is $\geq$ 1500 cells/uL and the platelet count is $\geq$ 100,000/uL. Initiation of a new cycle will be delayed for a maximum of two weeks until these values are achieved. Patients who fail to recover adequate counts within a two week delay will be removed from study

5.1.2 In case of neutropenic fever, grade 4 thrombocytopenia, or delay of a cycle for over 7 days, the carboplatin dose will be decreased by 25% for subsequent cycles. For a second episode of febrile neutropenia, G-CSF should be given with the next cycle. For an episode of febrile neutropenia despite dose reduction and G-CSF, protocol treatment should be discontinued.

### 5.2 Non-Hematologic Toxicity

5.2.1 Neuropathy
Grade 2 or greater peripheral neuropathy will require a delay in carboplatin therapy until recovery to grade 1.

5.2.2 Nephrotoxicity
If the creatinine is not within institutional normal limits on the day of a carboplatin or bevacizumab dose, carboplatin and bevacizumab therapy will be held. It may be re-instituted at full dose when the creatinine recovers. The patient may continue to receive trastuzumab.

5.2.3 Cardiotoxicity
If the patient develops clinical grade 3-4 cardiotoxicity, the herceptin and bevacizumab therapy will be discontinued permanently. The carboplatin therapy may continue.

5.2.4 Hypertension
Bevacizumab should be permanently discontinued in patients with hypertensive crisis. Temporary suspension is recommended in patients with severe (grade 3-4) hypertension that is not controlled with medical management. Once controlled to grade 0-1, bevacizumab may be reinstituted. However, if hypertension worsens to grade 3-4, bevacizumab should be discontinued permanently.

5.2.5 Hemorrhage
Patients with serious hemorrhage i.e., requiring medical intervention, should have bevacizumab treatment discontinued permanently and receive aggressive medical management.

5.2.6 Proteinuria

Bevacizumab should be permanently discontinued in patients with nephrotic syndrome ($\geq$ 3gm proteinuria/24 hours). For patients with worsening proteinuria (as defined by 1+ or greater protein by urine dipstick), bevacizumab therapy should be held and a 24 hour urine collection should be obtained. For patients with greater than 2 grams of proteinuria/24 hours, bevacizumab should be held and can be re-instituted only when proteinuria is less than 2 grams/24 hours. Patients with moderate to severe proteinuria should be monitored regularly until improvement and/or resolution. If proteinuria worsens (defined as proteinuria $\geq$ 2 grams/24 hours) with the reinstitution of bevacizumab, it should be discontinued permanently.

## 6. AGENT FORMULATION AND PROCUREMENT

### 6.1 Carboplatin

6.1.1 Product description: Carboplatin is a second generation tetravalent organic platinum compound. Like cisplatin, carboplatin produces predominantly interstrand DNA crosslinks rather than DNA-protein crosslinks and is cell-cycle non-specific.

6.1.2 Solution preparation: Add 5, 15, or 45 mL of sterile water, normal saline or 5% dextrose to the 50, 150 or 450 mg vials, respectively. The resulting solution contains 10 mg/mL. The desired dose is further diluted, usually in 5% dextrose.

6.1.3 Storage requirements: Intact vials are stored at room temperature protected from light.

6.1.4 Stability: When further diluted in glass or polyvinyl plastic to a concentration of 500 mg/mL, solutions have the following stability: in normal saline 8 hours at 25°, 24 hours at 5°.

6.1.5 Route of administration: Intravenous administration over 30 minutes on day 1 of every 3 week cycle.

6.1.6 Adverse effects:

Hematologic: thrombocytopenia, neutropenia, leukopenia, more pronounced in patients with compromised renal function

Gastrointestinal: nausea and vomiting, treatable with moderate doses of antiemetics, anorexia, diarrhea and constipation

Dermatologic: rash, urticaria

Hepatic: abnormal liver function testes, usually reversible with standard doses

Neurologic: rarely peripheral neuropathy is seen

Renal: elevations in serum creatinine, BUN; electrolyte loss

Other: asthenia, pain, flu-like symptoms

6.2 Bevacizumab

6.2.1 Product description: Recombinant humanized monoclonal antibody which binds to the vascular endothelial growth factor (VEGF).

6.2.2 Solution preparation: Opened vials must be used within 8 hours. The calculated dose should be placed in a sterile, empty IV bag and diluted with a sufficient amount of 0.9% NS injection to obtain a final volume of 100 mL.

6.2.3 Storage requirements: Vials should be refrigerated at 2-8° C and should remain refrigerated until use.

6.2.4 Stability: Once bevacizumab has been added to a bag of sterile saline, the solution must be administered within 8 hours.

6.2.5 Route of administration: Administered as a continuous IV infusion of 10 mg/kd every 3 weeks. First dose of bevacizumab should be administered over 90 minutes. If the first infusion is tolerated well, the second dose can be delivered over 60 minutes. If the second dose is tolerated well, then all subsequent doses can be delivered over 30 minutes.

6.2.6 Adverse effects:

Hematologic: arterial and venous thromboembolism, CNS hemorrhage, GI bleeding, epistaxis, pulmonary hemorrhage

Musculoskeletal: arthralgias, chest pain

Cardiovascular: hypertension (including hypertensive crisis), hypotension, decrease in cardiac function, pericardial effusion, tamponade

Renal: proteinuria, nephrotic syndrome

Reproductive: fertility impairment of unknown duration

Skin: rash, desquamation, urticaria, delay in wound healing

Gastrointestinal: nausea, colitis, intestinal obstruction, vomiting, bowel perforation

Pulmonary: dyspnea

Constitutional: fevers, chills, rigors, headaches, asthenia, infection without neutropenia

Hepatic: reversible and marked elevations of LFTs

6.3 Trastuzumab

6.3.1 Product description: A recombinant DNA-derived humanized monoclonal antibody against a transmembrane receptor protein structurally related to an epidermal growth factor receptor.

6.3.2 Solution preparation: A vial of trastuzumab is reconstituted with 20 mL of BWFI, as supplied. This yields 21 mL of a multi-dose solution containing 21 mg/mL of trastuzumab. The calculated volume needed to deliver the desired dose is withdrawn and added to a polyethylene bag containing 250 mL of 0.9% NS.

6.3.3 Storage requirements: Vials of trastuzumab are stable at 2-8°C prior to reconstitution with the expiration date stamped on the vial.

6.3.4 Stability: A vial of trastuzumab reconstituted with BWFI is stable for 28 days after reconstitution when stored refrigerated at 2-8°C.

6.3.5 Route of administration: The calculated dose of trastuzumab (8 mg/kg for dose 1 and 6 mg/kg for all subsequent doses) is diluted in 250 mL of 0.9% NS, and initially administered as a 90 minute infusion. If initial trastuzumab infusions are well-tolerated, subsequent infusion periods can be shorted to 30 minutes. Trastuzumab is given every 3 weeks.

6.3.6 Adverse effects:

Infusional: chills, fever, nausea, vomiting, rigors, pain, headache, dizziness, dyspnea, rash

Cardiac: congestive heart failure

Constitutional: generalized pain, abdominal pain, back pain, myalgia, weakness, rash, pruritis, urticaria, diarrhea

Gastrointestinal: diarrhea

## 7. CORRELATIVE/SPECIAL STUDIES

7.1 Biospecimen Processing

As part of the methylation analysis of tumor, paraffin embedded tissue will be required. Pathology reports along with either a representative block of tumor tissue or five 20 micron unstained slides and ten 5 micron slides will be collected. Specimens should be sent to:

Rita Nanda, M.D.
The University of Chicago Medical Center
5841 S. Maryland Ave., MC 2115
Room I-319
Chicago, IL 60637-1470
Attn: Lise Sveen

7.2 Methylation Analysis

We will use Methylation Specific PCR (MSP) to assess methylation status of the tumor. This assay will be performed according to the protocol established in the laboratory of Dr. Funmi Olopade at the University of Chicago.

7.3 Immunohistochemical Analysis

VEGF expression in the tumor will be determined by immunohistochemical analysis. The antibody against VEGF is commercially available and staining will be performed as previously described. All slides will be read independently by two pathologists. Each tumor will be stained in duplicate. The tumors will be scored using a 0-3+ scale as has been previously described. The final score will be a composite of the two independent reviewers scores.

## 8. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

| | Pre-Study | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 | Wk 7 | Wk 8 | Wk 9 | Wk 10 | Wk 11 | Wk 12 | Off Study[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carboplatin | | X | | | X | | | X | | | X | | | |
| Bevacizumab | | X | | | X | | | X | | | X | | | |
| Trastuzumab | | X | | | X | | | X | | | X | | | |
| Informed consent | X | | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | | |
| Concurrent meds | X | X---------- | | | | | | | | | | | ----------X | |
| Physical exam | X | X | | | X | | | X | | | X | | | X |
| Vital signs | X | X | | | X | | | X | | | X | | | X |
| Height | X | | | | | | | | | | | | | |
| Weight | X | X | | | X | | | X | | | X | | | X |
| Performance status | X | X | | | X | | | X | | | X | | | X |
| CBC w/diff, plts | X | X | | | X | | | X | | | X | | | X |
| Serum chemistry[a] | X | X | | | X | | | X | | | X | | | X |
| Urinanalysis | X | X | | | X | | | X | | | X | | | X |
| EKG | X | | | | | | | | | | | | | |
| MUGA | X | | | | | | | | | | | | | |
| Adverse event evaluation | | X---------- | | | | | | | | | | | ----------X | X |
| Tumor measurements | X | Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease. | | | | | | | | | | | | X[c] |
| B-HCG | X[b] | | | | | | | | | | | | | |
| IHC for ER/PR status | X | | | | | | | | | | | | | |
| IHC or FISH for HER2/neu status | X | | | | | | | | | | | | | |
| Tumor Block Collection | X[d] | | | | | | | | | | | | | |

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Serum pregnancy test (women of childbearing potential).
c: Four week treatment cycles continue while patient is on study.
d: To be sent to Rita Nanda within eight weeks of starting on trial.

## 9. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be evaluated for response after the first two cycles (six weeks) and every two to three cycles (six to nine weeks) thereafter. In addition to a baseline scan, confirmatory scans should also be obtained four to nine weeks following initial documentation of objective response.

### 9.1. Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

#### 9.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$20 mm with conventional techniques (CT, MRI, x-ray) or as $\geq$10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

#### 9.1.2 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

#### 9.1.3 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

### 9.1.4 Non-target lesions

All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

## 9.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If they are used, they should have progressed since the time of radiation treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

**Clinical lesions.** Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

**Chest x-ray.** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

**Conventional CT and MRI.** These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

**Ultrasound (US).** When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

**Tumor markers**. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

## 9.3 Response Criteria

### 9.3.1 Evaluation of target lesions

Complete Response (CR):Disappearance of all target lesions

| | |
|---|---|
| Partial Response (PR): | At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD |
| Progressive Disease (PD): | At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions |
| Stable Disease (SD): | Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started |

### 9.3.2 Evaluation of non-target lesions

| | |
|---|---|
| Complete Response (CR): | Disappearance of all non-target lesions and normalization of tumor marker level |
| Incomplete Response/ Stable Disease (SD): | Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits |
| Progressive Disease (PD): | Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions |

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair). Note: If tumor markers are initially above the upper normal

limit, they must normalize for a patient to be considered in complete clinical response.

### 9.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1).

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Incomplete response/SD | No | PR |
| PR | Non-PD | No | PR |
| SD | Non-PD | No | SD |
| PD | Any | Yes or No | PD |
| Any | PD | Yes or No | PD |
| Any | Any | Yes | PD |

Note:

X Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

X In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

## 9.4 Confirmatory Measurement/Duration of Response

### 9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4-9 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks (see section 9.3.3).

### 9.4.2 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

### 9.4.3 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

## 10. REGULATORY AND REPORTING REQUIREMENTS

Per University of Chicago Cancer Center Guidelines, this protocol will be classified as moderate risk. As such the patients enrolled to this study will be regularly discussed as a part of the weekly Genitourinary Oncology Conference. The discussion will include tumor response and toxicity. A Data and Safety Monitoring worksheet will be completed at this conference (Appendix E). Twenty percent of research charts will be audited annually.

Adverse event (AE) reporting for this study is via MedWatch. The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 3.0 will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTC version 3.0. A more complete list of adverse events that have occurred or might occur can be found in Section 5.2. A copy of the CTC version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

## 10.1 Expedited Adverse Event Reporting

### 10.1.1 Expedited Reporting Guidelines

| UNEXPECTED EVENT | | EXPECTED EVENT | |
|---|---|---|---|
| **GRADES 2 – 3**<br>**Attribution of Possible, Probable or Definite** | **GRADES 4 and 5**<br>**Regardless of Attribution** | **GRADES 1 - 3** | **GRADES 4 and 5**<br>**Regardless of Attribution** |
| Expedited report within 10 working days.<br><br>(Grade 1 Adverse Event Expedited Reporting NOT required.) | Report by phone to UCCRC Clinical Trials Office within 24 hrs. Expedited report to follow within 10 working days.<br><br>This includes all deaths within 30 days of the last dose of treatment regardless of attribution.<br><br>Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days. | Adverse Event Expedited Reporting NOT required. | Expedited report, within 10 working days.<br><br>This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.<br><br>Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.<br><br>Grade 4 Myelosuppression not to be reported, but should be submitted as part of study results. Other Grade 4 events that do not require expedited reporting would be specified in the protocol. |

- For **Hospitalization** only – Any medical event equivalent to CTC Grade 3, 4, or 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.

- Telephone reports to the University of Chicago Cancer Center Clinical Trials Office (773-702-3116) by the end of the business day when investigator and/or research study nurse becomes aware of the event. Events occurring after business hours will be reported to the PDMO by 12 pm (noon) the next business day.

- The following information is required when calling in the event:
  - -Reporter's Name and Telephone Number
  - -Patient Initials and Medical Record Number
  - -IRB Protocol Number
  - -PI of Study
  - -Attending Physician
  - -Date of Event
  - -Description of Event (including grade of the event and if the event required hospitalization)

- E-mail is sent to the research nurse, attending physician and PI of the study informing them that ADR notification has been received.

- A completed MedWatch form (FDA form 3500A) must be sent to the University of Chicago Cancer Center Clinical Trials along with the University of Chicago's IRB Adverse Event Form within **5 working days of event occurrence**. The UC IRB Adverse Event form is available on-line at: http://ors.bsd.uchicago.edu/HS/newirbforms. This form must be typed. Once the forms are completed forward the original to the study PI in the pink SAE folder. The PI will then review, sign and place folder in QA coordinator's box. A weekly report of delinquent or pending documents will be forwarded to Denise Friesema, RN (U of C Events Only). All delinquent reporting (greater than 10 days from event occurrence) must include documentation of reason for delinquency and may require implementation of an action plan.

- Once the appropriate AE documents have been received, the University of Chicago Cancer Center Clinical Trials forwards these to the IRB and a copy will be forwarded to the appropriate Research Nurse.

10.1.2 Agent-Specific Expected Adverse Events List

The list below guides the investigator in determining which AEs require expedited reporting. Those AEs that do not require expedited reporting are reported in routine study data submissions.

- Skin irritation including pruritis, erythema, induration, blisters
- Fluid retention
- Nausea and/or vomiting
- Fatigue

10.1.3 Forms

The UC IRB Adverse Event form is available on-line at: http://ors.bsd.uchicago.edu/HS/newirbforms. The MedWatch form is available at http://www.fda.gov/medwatch/safety/3500a.pdf.

## 10.2 Multicenter Guidelines

### 10.2.1 Registration

All patients must be registered with the University of Chicago Registrar Office (773-834-7839) prior to the commencement of treatment. Confirm all selection criteria listed in Section 3.0, then call the Registrar Office with the following information:

Provider of information
Study # and Institution
Treating Physician
Patient name and hospital ID number
Patient's zip code of residence
Date of signed informed consent
Race, gender, date of birth of patient
Diagnosis and date of initial diagnosis

10.2.2 **Data Submission**

| | |
|---|---|
| On-study | Submit specific registration packet and source documentation **prior** to registration. |
| Weekly | Fax weekly flow sheets and toxicity and chemotherapy summary forms by noon on Friday of each week, for review at the weekly Phase II Conference. |
| Evaluations | At each evaluation as specified in the protocol, complete the extent of disease form, specify response (CR, PR, SD, PD) on the flow sheet and submit source documentation of the response (CT, x-ray, physical exam). |
| Off-study | Submit a flow sheet documenting date of treatment completion. |
| Follow-up | Submit follow-up and notification of death form every 3 months, documenting disease progression, second line therapy, last date known alive or date of death. |

10.3 **Data and Safety Monitoring**

Data Safety and Monitoring will occur at the weekly University of Chicago Phase II Consortium meetings, which are lead by senior level medical oncologists. At each meeting, all active Phase II Consortium studies will be reviewed for safety and progress toward completion. Toxicities and adverse events will be reviewed at each meeting and a Data Safety and Monitoring form will be filled out for each protocol and signed by either the principal investigator, the Chairman of the Phase II Consortium or by his designate if the chairman is not available.

## 11. STATISTICAL CONSIDERATIONS

### 11.1 Study Design/Endpoints

The primary endpoint for this trial is response rate.

A Simon's minimax two-stage design will be employed. The target response rate and lower bound response rate are 45% and 25% respectively for each of the two treatment arms. Using a significance of $\alpha$=0.05 and $\beta$=0.2, the total number of patients needed for each arm is 36. The two arms will accrue independently, and the early stopping rules will apply to each arm, independent of the status of the other arm. In the first stage, 17 patients are treated (for each arm). If 5 or more patients respond, then another 19 patients will be added to that arm, for a total of 36 patients per arm. The patients will be followed to disease progression. If one arm of the study closes for poor response rate, the other arm will continue to accrue until it is either closed for poor response or completes accrual. If fewer than 12 responses are observed in either arm by the end of the second stage, the regimen will be considered of low interest in that arm, and further studies involving this combination will not be planned.

### 11.2 Sample Size/Accrual Rate

The planned sample size is 72 patients total. With an expected accrual rate of 6 patients per month, the total accrual time will be approximately one year.

### 11.3 Analysis of Secondary Endpoints

The secondary endpoints of this trial will be time to disease progression, correlation of response rate to tumor *BRCA1* promoter hypermethylation, and correlation of response rate to tumor VEGF expression. The time to disease progression will be estimated using the Kaplan-Meier method with a 95% confidence interval. The possible risk factors (e.g. *BRCA1* methylation) will be compared for time to progression using the log-rank test. For multivariate analysis, the proportional hazards Cox model will be applied to investigate potential prognostic factors, such as *BRCA1* methylation status and tumor VEGF expression on the time to progression. The adjusted p-values of the odds ratios and the adjusted 95% confidence interval will be reported.

### 11.5 Reporting and Exclusions

11.5.1 **Evaluation of toxicity.** All patients will be evaluable for toxicity from the time of their first treatment with carboplatin, bevacizumab and trastuzumab.

11.5.2 **Evaluation of response.** All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the following categories: 1)

complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.] All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

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## APPENDIX A

### Performance Status Criteria

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work). | 80 | Normal activity with effort; some signs or symptoms of disease. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60 | Requires occasional assistance, but is able to care for most of his/her needs. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance. |
| | | 30 | Severely disabled, hospitalization indicated. Death not imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

**THE UNIVERSITY OF CHICAGO**
**THE DIVISION OF BIOLOGICAL SCIENCES**
**THE UNIVERSITY OF CHICAGO HOSPITALS**

**Consent By Subject for Participation in Research Protocol**

**Protocol Number:** _____ **Patient Name:** _____

**Patient Medical History Number:** _____

Title of Protocol: A Phase II Study of Carboplatin plus Bevacizumab (+/- Trastuzumab) in Patients with Hormone Receptor Negative Metastatic Breast Cancer

**Doctors Directing Research**: Rita Nanda, M.D. Phone: (773) 834-2756
Funmi Olopade, M.D. Phone: (773) 702-1632

You are being asked to participate in a research study. The doctors at the University of Chicago Hospitals and The Division of Biological Sciences study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent. This consent form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your friends and family.

You are being asked to take part in this study because you have breast cancer that cannot be cured by surgery and/or radiation alone. Your breast cancer is estrogen receptor negative, which means that it will not shrink by blocking the effects of estrogen in your body. When tumors are estrogen negative, they usually are faster growing and harder to shrink with the drugs we have now.

## WHY IS THIS STUDY BEING DONE?

The purpose of this study is to find out what effects (good and bad) the combination of carboplatin (a chemotherapy drug) and bevacizumab (a therapy which targets a protein that is commonly positive in estrogen receptor negative breast tumors) has on your breast cancer and you. If your breast tumor is positive for the Her2/*neu* protein, you will also get trastuzumab in addition to the carboplatin and bevacizumab. Trastuzumab is a drug, which like bevacizumab, is a drug that works by targeting a protein that is produced by some breast tumors. This research is being done because currently no curative treatment exists for recurrent or metastatic (spread of cancer cells from one area of the body to

another) breast cancer. The purpose of this study is to find out how well the combination of carboplatin and bevacizumab (and trastuzumab for those half of patients with tumors that are positive for the Her2/*neu* protein) can shrink tumors, and how serious the side effects are.

Both carboplatin and bevacizumab are FDA-approved drugs, but they are not approved for use in breast cancer, therefore the use of this combination in breast cancer is considered experimental. Trastuzumab is FDA-approved for the treatment of breast cancers that are positive for the Her2/*neu* protein, but is not FDA approved to be given in combination with carboplatin and bevacizumab. All three of these drugs have been given to breast cancer patients, but not together. Each drug on its own has not been as good at shrinking tumors in women with breast cancer as we would have liked, but the reason we are doing this study is because we think that the combination of the two (or three) will work well in people who have estrogen receptor negative tumors.

A second purpose of the study is to find out if certain changes in cancer affect the response to these chemotherapy drugs. Some breast cancers turn off a gene that repairs damage to cancer cells. We will examine your tumor (that was removed at the time of your diagnosis) to see if it has this change or not, and see if that matters for how well the chemotherapy shrinks the cancer.

## HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

There will be a total of 72 patients in this study. Everyone will receive carboplatin and bevacizumab. Only the half of patients with Her2/*neu* positive tumors will receive trastuzumab in addition to the carboplatin and bevacizumab.

## WHAT IS INVOLVED IN THE STUDY?

If you agree to take part in this study, you will have a physical exam by your doctor and blood tests will be done to determine if you meet the trial requirements. These blood tests will be done before you start the study and every three weeks while taking carboplatin chemotherapy to be sure you are not experiencing any unseen side effects. The total amount of blood taken each time is about half a tablespoon. If you are a woman who can have children, a pregnancy test (1 teaspoon of blood) will be done.

In most cases a CT (computed tomography) scan or MRI (magnetic resonance imaging) and possibly chest x-ray, will be performed to measure the amount and size of tumors (cancer) in your body. A CT scan is a computerized x-ray that is transmitted onto a computer that gives your doctor clearer pictures of the inside of your body. An MRI is a test that uses magnetic pulses to take pictures of the inside of your body. A CT scan, MRI and x-ray are routine procedures used to help doctors monitor your cancer. You will have a CT scan or MRI ordered before starting the study drugs, after six weeks of receiving the

study drugs, and then every nine weeks after that while you are receiving the study drugs. This will allow you and your doctors to know whether or not the cancer is shrinking.

You will see your doctor every 3 weeks. Each visit will consist of your doctor asking questions about you and any side effects you may be having. Your doctor will also perform a physical examination. At each visit you will have your blood drawn as described above.

All of the above procedures are considered part of standard care, that is, they would be done if you were receiving chemotherapy even if it were not part of a clinical trial.

The study drugs will be given on an outpatient basis. The drugs will be given by vein. The carboplatin will be given by vein over 30 minutes every three weeks. The bevacizumab will also be given by vein every three weeks. This first dose of bevacizumab will take 90 minutes. If you tolerate that well, the second will take 60 minutes. And, if you tolerate that well, all of the rest will only take 30 minutes. If your tumor is positive for Her2/*neu*, you will also receive trastuzumab by vein every three weeks. The first dose of trastuzumab will take 90 minutes. If you tolerate it will, all the rest will only take 30 minutes. Every time you get a dose of carboplatin, bevacizumab and trastuzumab you start a new "cycle." Because you will be given a dose of these drugs every three weeks, a new cycle starts every three weeks.

As a part of this clinical trial, samples of your cancer tissue (from a surgery or biopsy which has already been done and from which a piece of the cancer was saved—no new biopsy will be performed for this study) will be sent to the laboratory of Dr Funmi Olopade at the University of Chicago. The tissue will be tested for an alteration called "*BRCA1* promoter methylation." This is a change occurring in some cancer cells that may affect the cancer cell's ability to repair damage. We will try to determine whether or not this change affects how your cancer responds to chemotherapy. Because this is still a research test, and not approved for clinical use, neither you nor your doctor will be notified of the results of this test. It will have no effect on the treatment you receive in this clinical trial. The cancer tissue will not be shared with anybody else or used for any other tests. Unused tissue will be returned to the pathology department it came from.

**HOW LONG WILL I BE IN THE STUDY?**

You may remain on study as long as your disease is responding to the drug or your disease is stable and your side effects are not too bad. The decision about how long to continue if your disease is not getting worse is up to you and your doctor.

The study drugs will be stopped if
Your cancer gets worse even though you are receiving the study drugs;
Side effects of the study drugs are too hard on you or too dangerous for you;
You develop another illness or condition which would not allow you to continue;
You do not comply with study procedures;

New information about the drug combination becomes available and this information suggests the drugs will be ineffective or unsafe for you.

You can also choose to stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

**WHAT ARE THE RISKS OF THE STUDY?**

While on the study, you are at risk for experiencing side effects including those listed below. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict. Other drugs will be given to make side effects less serious and uncomfortable. Many side effects go away shortly after carboplatin, bevacizumab and trastuzumab are stopped, but in some cases side effects can be serious or long-lasting, permanent, or even fatal.

Common side effects:

General symptoms: Fatigue, flu-like symptoms, abdominal pain
Bleeding: Nose bleeds
Gastrointestinal: Decreased appetite, nausea, vomiting, diarrhea
Cardiac: High blood pressure
Hematologic: Low blood counts including anemia (which may require blood transfusions or the use of injections to raise the red blood cell count), low white blood cells (which leads to an increased risk of infection), low platelet counts (which can lead to an increased risk of bleeding).

Less common side effects:

Skin: Hair loss, rash
Electrolyte: Loss of potassium and magnesium in the urine
Liver: High levels of some blood tests of liver function (usually with no symptoms)
Kidneys: Kidney damage, blood and protein in the urine, swelling

Rare but severe:

Hemorrhage: Life-threatening bleeding in the brain and the intestines
Heart: Heart failure, Uncontrollable high blood pressure which can lead to death
Blood: Leukemia (cancer of the white blood cells)
Allergy: Severe allergic reactions with shortness of breath and high or low blood pressure
Lungs: Shortness of breath
Kidneys: Kidney damage that can lead to death or require dialysis

Reproductive Risks: Because the effect of the agents in this study on an unborn baby are not certain, you should not become pregnant while on this study. You should not breast feed your baby while on this study. Ask about counseling and more information about preventing pregnancy.

Blood drawing risks: There may be bruising, bleeding or inflammation at the sites where blood samples are taken. Care will be provided to avoid these complications.

MRI risks: Risks from the MRI test may include an allergic reaction to the contrast dye and a feeling of claustrophobia (fear of closed-in spaces) that can make you feel anxious and nervous. You will be required to rest still in a dark and closed space during the time of testing. If you have a fear of closed spaces (claustrophobia) you may not be able to undergo this test or you may require additional medications to prepare you for taking the MRI test. The radiologist (a doctor specializing in the field of radiology) who will conduct the MRI test will explain the procedure to you in detail.

CT risk: Risks from the CT scans may include an allergic reaction to the contrast dye or kidney problems from the dye. Also, you might have problems with claustrophobia, which may make it hard to get the test or require you to have additional medicines before you have the test. The doctor who does the test will explain everything to you in detail.

Treatment Risks: One risk of the treatment is that the combination of all of the drugs might have worse side effects than any one of the drugs alone. There is also no guarantee that the drugs will work for you and your tumor.

## WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct benefit to you. The benefit of carboplatin and bevacizumab at this dose given to subjects with breast cancer is not known. However, the information gained from this study could benefit other individuals with this type of disease in the future.

## WHAT OTHER OPTIONS ARE THERE?

Instead of being in this study, you have these options:

Standard chemotherapy (chemotherapy with approved drugs).
Other experimental drugs.
Comfort care only, where treatments are directed only at reducing symptoms, relieving suffering and maximizing comfort, dignity, and control. In comfort care only, treatment is not directed at curing, slowing, or reversing your disease.

Please talk to your doctor about these and other options.

## WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. Your medical records will contain your name, address, and medical history number and will be available to and kept secure by the study doctor, research nurse, and data coordinator. Neither your name nor other personally identifying information will be used in any publication resulting from this research study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Certain organizations, including qualified representatives of the University of Chicago Protocol and Data Management Office, the University of Chicago Institutional Review Board, National Cancer Institute, Food and Drug Administration, and Genentech, the pharmaceutical company that makes bevacizumab, may inspect and/or copy your research records for quality assurance and data analysis.

## WHAT ARE THE COSTS?

You and your insurance company will be responsible for the cost of the carboplatin, trastuzumab, and all labs, x-rays, scans, clinic visits, physical exams, biopsies and any hospital admissions. Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems. The bevacizumab will be provided by Genentech, the pharmaceutical company that makes the drug.

The special research studies performed on your cancer tissue biopsy will not be charged to you or your insurance company.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

You or your insurance company will be billed for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

## WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information that may affect your health, welfare, or willingness to stay on this study.

Data Safety and Monitoring, which is when doctors, nurses and other research personnel review the data from the study to ensure that it is safe to continue, will occur on a weekly basis for this study. We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay on this study.

**WHERE CAN I GET MORE INFORMATION?**

You can contact the study chair, Rita Nanda, M.D. at (773) 834-2756.

You may call the Cancer Information Service at:
1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web Sites.
cancerTrials: comprehensive clinical trials information
http://www.cancer.gov/clinical_trials/

CancerNet™: accurate cancer information including PDQ
http://www.cancer.gov/cancer_information/.

You will get a copy of this form. You can also request a copy of the protocol (full study plan).

If you have questions concerning my rights in connection with the research, you can contact the University of Chicago Institutional Review Board (which is a group of people who review the research to protect your rights), at (773) 702-6505.

## AGREEMENT TO CONSENT

The research project and treatment procedures associated with it have been explained to me. The experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available at the University of Chicago Hospitals. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law the records of this research may be reviewed by representatives of the University of Chicago Protocol and Data Management Office, representatives of the University of Chicago Institutional Review Board, Eli Lilly (the manufacturer of gemcitabine) and by applicable government agencies including the Federal Food and Drug Administration and National Cancer Institute.

In the event of physical injury resulting from this research, the University of Chicago Hospitals will provide me with free emergency care, if such care is necessary. If I wish, the University of Chicago Hospitals will provide non-emergency care, but the Medical Center assumes no responsibility to pay for such care or to provide me with financial compensation.

I, the undersigned, hereby consent to participate as a subject in the above described research project conducted at the University of Chicago. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, including if a research related injury occurs, I can contact Rita Nanda, M.D. at (773) 834-2756. If I have questions concerning my rights in connection with the research, I can contact the Institutional Review Board, at (773) 702-6505.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

SUBJECT_____DATE _____TIME_____AM/PM(circle)

WITNESS_____ DATE _____TIME_____AM/PM(circle)

PHYSICIAN_____ DATE _____TIME_____AM/PM(circle)

# APPENDIX III

## *BRCA1* Promoter Methylation Confers Sensitivity to Cisplatin *in vitro.*

Nanda R, Dignam J, Collins C, Patel B, Dolan ME, Olopade OI.
University of Chicago, Chicago, IL.

**Background**: Several groups have demonstrated that women with *BRCA1* mutations are more likely to have breast cancers that are hormone receptor and HER2/*neu* negative. Our lab has previously demonstrated that *BRCA1* promoter methylation occurs to some degree in 30% of all sporadic tumors, and up to 50% of high-grade hormone receptor negative tumors, making it much more common than germline mutation. Given the role of *BRCA1* in DNA repair, it is likely that cells deficient in BRCA1 secondary to promoter methylation will have an increased sensitivity to DNA damaging agents, as has previously been demonstrated in cells deficient in BRCA1 secondary to mutation. The role of *BRCA1* methylation in determining chemosensitivity is not yet known.

**Methods**: Using an *in vitro* cell line model, the relative sensitivity of *BRCA1* methylated, mutated and competent breast cancer cell lines was determined. Four breast cancer cell lines were used to determine relative sensitivity: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with doses of cisplatin between 0.25 uM and 350 uM. Each cell line was exposed to escalating doses of cisplatin in triplicate on three separate times. Untreated cells served as a control. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival and apoptosis were determined by flow cytometry using FACS DiVa. FlowJo FACS analysis software (version 6.1.1) was used to generate percent apoptotic and live cells. Cells that were negative for both Annexin-V and DAPI were considered live, and cells that were positive for Annexin-V and negative for DAPI were considered apoptotic. Each experiment was normalized to its own dose 0 average, and percent live vs dose and percent apoptotic vs dose curves were constructed. IC50 values were calculated from sigmoidal dose response curves.

**Results**: The IC50 values for the UACC-3199 and HCC-1937 cells were 16.7 uM and 78.0 uM, respectively. The IC50 values for MCF-7 and MDA-MB-231 cells were not reached, even at a dose of 350 uM. Peak percentage of apoptotic cells observed for the UACC-3199 was 40% at a cisplatin concentration of 50 uM. Peak percentage of apoptotic cells observed for the HCC-1937, MCF-7 and MDA-MB-231 cells were 20%, 16% and 21% at cisplatin concentrations of 100 uM, 350 uM, and 350 uM, respectively.

**Discussion**: Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are more sensitive to cisplatin than *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary promoter methylation are also highly sensitive to cisplatin. As *BRCA1* methylation occurs in almost one-half of high-grade hormone receptor negative tumors, it represents a potential therapeutic target in the treatment of a subset of hormone receptor negative breast cancers.